# NiceProt View of Swiss-Prot: P13810



*Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.*

## Entry information

| | |
|---|---|
| Entry name | **E2AA_ECOLI** |
| Primary accession number | **P13810** |
| Secondary accession numbers | None |
| Entered in Swiss-Prot in | Release 13, January 1990 |
| Sequence was last modified in | Release 13, January 1990 |
| Annotations were last modified in | Release 44, July 2004 |

## Name and origin of the protein

| | |
|---|---|
| Protein name | **Heat-labile enterotoxin IIA, A chain [Precursor]** |
| Synonym | **LT-IIA** |
| Gene name | None |
| From | Escherichia coli [TaxID: 562] |
| Taxonomy | Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia. |

## References

[1] SEQUENCE FROM NUCLEIC ACID.
MEDLINE=88032841;PubMed=2822667 [NCBI, ExPASy, EBI, Israel, Japan]
Pickett C.L., Weinstein D.L., Holmes R.K.;
"Genetics of type IIa heat-labile enterotoxin of Escherichia coli: operon fusions, nucleotide sequence, and hybridization studies.";
J. Bacteriol. 169:5180-5187(1987).

## Comments

- ***FUNCTION***: The biological activity of the toxin is produced by the A chain, which activates intracellular adenyl cyclase.
- ***SUBUNIT***: Heterohexamer of one A chain and of five B chains.

## Copyright

**Cross-references**

| | |
|---|---|
| EMBL | M17894; AAA24093.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence] |
| PIR | A29831; A29831. |
| HSSP | P06717; 1LTG. [HSSP ENTRY / PDB] |
| InterPro | IPR001144; Enterotoxin_A.<br>Graphical view of domain structure. |
| Pfam | PF01375; Enterotoxin_a; 1.<br>Pfam graphical view of domain structure. |
| PRINTS | PR00771; ENTEROTOXINA. |
| ProDom | [Domain structure / List of seq. sharing at least 1 domain] |
| HOBACGEN | [Family / Alignment / Tree] |
| BLOCKS | P13810. |
| ProtoNet | P13810. |
| ProtoMap | P13810. |
| PRESAGE | P13810. |
| DIP | P13810. |
| ModBase | P13810. |
| SMR | P13810; 996F311A32CABEAA. |
| SWISS-2DPAGE | Get region on 2D PAGE. |
| UniRef | View cluster of proteins with at least 50% / 90% identity. |

**Keywords**

**Enterotoxin**; **Signal**.

**Features**

Feature table viewer

| Key | From | To | Length | Description |
|---|---|---|---|---|
| SIGNAL | 1 | 18 | 18 | |
| CHAIN | 19 | 259 | 241 | Heat-labile enterotoxin IIA, A chain. |
| NP_BIND | 23 | 37 | 15 | NAD (*By similarity*). |
| ACT_SITE | 128 | 128 | | *By similarity*. |
| DISULFID | 203 | 215 | | *By similarity*. |

**Sequence information**

Length: **259 AA** [This is the length of the unprocessed precursor]

Molecular weight: **29242 Da** [This is the MW of the unprocessed precursor]

CRC64: **996F311A32CABEAA** [This is a checksum on the sequence]

```
        10         20         30         40         50         60
         |          |          |          |          |          |
MIKHVLLFFV FISFSVSAND FFRADSRTPD EIRRAGGLLP RGQQEAYERG TPININLYEH

        70         80         90        100        110        120
         |          |          |          |          |          |
ARGTVTGNTR YNDGYVSTTV TLRQAHLIGQ NILGSYNEYY IYVVAPAPNL FDVNGVLGRY

       130        140        150        160        170        180
         |          |          |          |          |          |
SPYPSENEFA ALGGIPLSQI IGWYRVSFGA IEGGMQRNRD YRGDLFRGLT VAPNEDGYQL
```

```
      190         200         210         220         230         240
       |           |           |           |           |           |
AGFPSNFPAW REMPWSTFAP EQCVPNNKEF KGGVCISATN VLSKYDLMNF KKLLKRRLAL

       250
        |
TFFMSEDDFI GVHGERDEL
```

P13810 in FASTA format

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BLAST submission on ExPASy/SIB or at NCBI (USA)

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

ScanProsite, MotifScan

Search the SWISS-MODEL Repository

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| --- | --- | --- | --- | --- |

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# WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Thursday, September 30, 2004

| Hide? | Set Name | Query | Hit Count |
|---|---|---|---|
| | *DB=USPT; PLUR=YES; OP=AND* | | |
| ☐ | L1 | (rlt or lt or lt-11a or lt11a or lt2 or rhlt or rh-lt or rh-lt-11).clm. | 330 |
| ☐ | L2 | (a near3 (domain or moiety or subunit or sub-unit or region or portion)).clm. | 10727 |
| ☐ | L3 | (b near3 (domain or moiety or subunit or sub-unit or region or portion)).clm. | 23369 |
| ☐ | L4 | L3 and l2 | 1909 |
| ☐ | L5 | L4 and l1 | 2 |
| ☐ | L6 | L4 and l1 | 2 |
| ☐ | L7 | L4 and l1 | 2 |
| ☐ | L8 | lt11a or lt11-a or lt-11a or (rhlt near2 2a) or r-hlt-2a or r-hlt-11-a or (heat near labile near2 (11a or 2a)) | 0 |
| ☐ | L9 | lt-11a or lt-lla | 0 |
| | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND* | | |
| ☐ | L10 | lt-11a | 0 |
| ☐ | L11 | L10 | 0 |
| ☐ | L12 | heat near2 labil$ | 3395 |
| ☐ | L13 | L12 same (2a or 11a or iia or ii-a or ll-a or 2-a) | 27 |

END OF SEARCH HISTORY

# WEST Search History

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| ☐ | L1 | (rlt or lt or lt-11a or lt11a or lt2 or rhlt or rh-lt or rh-lt-11).clm. | 330 |
| ☐ | L2 | (a near3 (domain or moiety or subunit or sub-unit or region or portion)).clm. | 10727 |
| ☐ | L3 | (b near3 (domain or moiety or subunit or sub-unit or region or portion)).clm. | 23369 |
| ☐ | L4 | L3 and l2 | 1909 |
| ☐ | L5 | L4 and l1 | 2 |
| ☐ | L6 | L4 and l1 | 2 |
| ☐ | L7 | L4 and l1 | 2 |
| ☐ | L8 | lt11a or lt11-a or lt-11a or (rhlt near2 2a) or r-hlt-2a or r-hlt-11-a or (heat near labile near2 (11a or 2a)) | 0 |
| ☐ | L9 | lt-11a or lt-lla | 0 |
| | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND* | | |
| ☐ | L10 | lt-11a | 0 |
| ☐ | L11 | L10 | 0 |
| ☐ | L12 | heat near2 labil$ | 3395 |
| ☐ | L13 | L12 same (2a or 11a or iia or ii-a or ll-a or 2-a) | 27 |
| ☐ | L14 | l4 and (mutant or mutation or mutagenesis or deletion or substitution or alteration or altered or modification or modified or insertion or inserted).clm. | 246 |
| ☐ | L15 | l14 and (coli or vibrio or cholera) | 40 |

END OF SEARCH HISTORY

# WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Thursday, September 30, 2004

| Hide? | Set Name | Query | Hit Count |
|---|---|---|---|
| | | *DB=USPT; PLUR=YES; OP=AND* | |
| ☐ | L1 | (ab5 or a-b-5 or ab-5) near10 ($toxin or toxi$) | 0 |
| ☐ | L2 | (ab5 or a-b-5 or ab-5).clm. | 5 |
| ☐ | L3 | (ab or a-b or ab).clm. same $toxin.clm. | 12 |
| ☐ | L4 | mutant.clm. same toxin.clm. | 69 |
| ☐ | L5 | achain or a-chain or (a near2 chain) | 30062 |
| ☐ | L6 | bchain or b-chain or (b near2 chain) | 8074 |
| ☐ | L7 | L6 same l5 | 2126 |
| ☐ | L8 | L7 same (mutant or mutation or mutagenesis or deletion or deleted or delete or substitute or substition or modification or insertion or insert or inserted or alter or alteration or altered or derivative or derived or detoxified or detoxification or detoxify or variant or variation or avirulent or a-virulent) | 763 |
| ☐ | L9 | L8 and (vibrio or mct or mlt or mlt11a or coli) | 476 |
| ☐ | L10 | L8 and (vibrio or mct or mlt or mlt11a or coli or cholera) | 480 |
| ☐ | L11 | L8 same (vibrio or mct or mlt or mlt11a or coli or cholera) | 68 |

END OF SEARCH HISTORY

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Generate Collection Print

L13: Entry 2 of 27 File: PGPB Sep 16, 2004

DOCUMENT-IDENTIFIER: US 20040181036 A1
TITLE: Mutant forms of cholera holotoxin as an adjuvant

Summary of Invention Paragraph:
[0008] Co-administration of CT with an unrelated antigen has been reported to result in the induction of concurrent circulating and mucosal antibody responses to that antigen (Mekalanos, J. J., et al., 1983 Nature, 306, 551-557). To minimize the occurrence of undesirable symptoms such as diarrhea caused by wild-type CT in humans, it would be preferable to use as an adjuvant a form of the CT holotoxin that has substantially reduced toxicity. Mutants of CT have been suggested as a means for achieving a more useful adjuvant. One way to rationally design mutant cholera toxin holotoxins (CT-CRMs) with substantially reduced toxicity is to identify and alter amino acid residues in the toxin molecule that are completely conserved in the family of cholera (CT) and related heat-labile enterotoxins (LT-I, LT-IIa and LT-IIb) of E. coli. Another rational way to generate mutant CT-CRMs with substantially reduced toxicity is to alter amino acid residues in the holotoxin molecule that have been identified as being important for NAD-binding based on the structural alignment of the CT backbone with the backbone of related toxins possessing ADP-ribosyl transferase enzyme activity such as diphtheria toxin (DT) and pertussis toxin (PT) (Holmes, R. K., "Heat-labile enterotoxins (Escherichia coli)" in Guidebook to Protein Toxins and their Use in Cell Biology, Montecucco, C. and Rappnoli, R., Eds., Oxford Univ. Press, Oxford, England (1997); and Holmes, R. K. et al, "Cholera toxins and related enterotoxins of Gram-negative bacteria", pp. 225-256 in Handbook of Natural Toxins: Bacterial Toxins and Virulence Factors in Disease, vol. 8, Moss. J., et al, Eds., Marcel Dekker, Inc., New York, N.Y. 1995).

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L13: Entry 4 of 27 File: PGPB Sep 9, 2004

DOCUMENT-IDENTIFIER: US 20040175723 A1
TITLE: TaqManTM-PCR for the detection of pathogenic E.coli strains

Detail Description Paragraph:
[0151] 43. Pickett, C. L., D. L. Weinstein, and R. K. Holmes. 1987. Genetics of type IIa heat-labile enterotoxin of Escherichia coli: operon fusions, nucleotide sequence, and hybridization studies. J. Bacteriol. 169:5180.

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**Heat-labile enterotoxin A chain precursor (LT-A, porcine) (LTP-A) [eltA] [Escherichia coli]** 258 AA align

Score = 427 bits (1099), Expect = e-119
Identities = 198/242 (81%), Positives = 222/242 (90%)

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Query: 17  YANDDKLYRADSRPPDEIKQSGGLMPRGQSEYFDRGTQMNINLYDHARGTQTGFVRHDDG 76
           YAN D+LYRADSRPPDEIK+SGGLMPRG +EYFDRGTQMNINLYDHARGTQTGFVR+DDG
Sbjct: 17  YANGDRLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNINLYDHARGTQTGFVRYDDG 76

Query: 77  YVSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVNDVLGAYSPHPDEQEVSALGG 136
           YVSTS+SLRSAHL GQ+ILSG+STYYIYVIATAPNMFNVNDVLG YSPHP EQEVSALGG
Sbjct: 77  YVSTSLSLRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDVLGVYSPHPYEQEVSALGG 136

Query: 137 IPYSQIYGWYRVHFGVLDEQLHRNRGYRDRYYSNLDIAPAADGYGLAGFPPEHRAWREEP 196
           IPYSQIYGWYRV+FGV+DE+LHRNR YRDRYY NL+IAPA DGY LAGFPP+H+AWREEP
Sbjct: 137 IPYSQIYGWYRVNFGVIDERLHRNREYRDRYYRNLNIAPAEDGYRLAGFPPDHQAWREEP 196

Query: 197 WIHHAPPGCGNAPRSSMSNTCDEKTQSLGVKFLDEYQSKVKRQIFSGYQSDIDTHNRIKD 256
           WIHHAP GCGN+ R+   +TC+E+TQ+L   +L EYQSKVKRQIFS YQS++D +NRI+D
Sbjct: 197 WIHHAPQGCGNSSRTITGDTCNEETQNLSTIYLREYQSKVKRQIFSDYQSEVDIYNRIRD 256

Query: 257 EL 258
           EL
Sbjct: 257 EL 258
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tr O66280 **Heat-labile enterotoxin A subunit [LTh a subunit] [Escherichia coli]** 258 AA align

Score = 427 bits (1098), Expect = e-119
Identities = 198/242 (81%), Positives = 222/242 (90%)

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           YAN DKLYRADSRPPDEIK+SGGLMPRG +EYFDRGTQMNINLYDHARGTQTGFVR+DDG
Sbjct: 17  YANGDKLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNINLYDHARGTQTGFVRYDDG 76

Query: 77  YVSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVNDVLGAYSPHPDEQEVSALGG 136
           YVSTS+SLRSAHL GQ+ILSG+STYYIYVIATAPNMFNVNDVLG YSPHP EQEVSALGG
Sbjct: 77  YVSTSLSLRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDVLGVYSPHPYEQEVSALGG 136

Query: 137 IPYSQIYGWYRVHFGVLDEQLHRNRGYRDRYYSNLDIAPAADGYGLAGFPPEHRAWREEP 196
           IPYSQIYGWYRV+FGV+DE+LHRNR YRDRYY NL+IAPA DGY LAGFPP+H+AWREEP
Sbjct: 137 IPYSQIYGWYRVNFGVIDERLHRNREYRDRYYRNLNIAPAEDGYRLAGFPPDHQAWREEP 196

Query: 197 WIHHAPPGCGNAPRSSMSNTCDEKTQSLGVKFLDEYQSKVKRQIFSGYQSDIDTHNRIKD 256
           WIHHAP GCGN+ R+   +TC+E+TQ+L   +L +YQSKVKRQIFS YQS++D +NRI+D
Sbjct: 197 WIHHAPQGCGNSSRTITDDTCNEETQNLSTIYLRKYQSKVKRQIFSDYQSEVDIYNRIRD 256

Query: 257 EL 258
           EL
Sbjct: 257 EL 258
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Updated Search

# WEST Search History

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DATE: Wednesday, September 29, 2004

| Hide? | Set Name | Query | Hit Count |
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| | | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND* | |
| ☐ | L1 | adp.ti. | 393 |
| ☐ | L2 | L1 and ((3$29 or 29$3 or 3$29$3) same position) | 32 |
| ☐ | L3 | L1 and (($29 or 29$3 or $29$3) same position) | 30 |
| ☐ | L4 | (ribosyl$ or adpribos$ or $ribosyltransferase or ribosyl-transferse or holotoxin) | 9612 |
| ☐ | L5 | L4 and (position near5 29) | 196 |
| ☐ | L6 | mutant.ti. and adjuvant$.ti. | 28 |
| | | *DB=EPAB,JPAB,DWPI; PLUR=YES; OP=AND* | |
| ☐ | L7 | 9702348 | 11 |
| | | *DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND* | |
| ☐ | L8 | e29 or glu-29 or glu29 | 879 |
| ☐ | L9 | L8 and l4 | 74 |
| ☐ | L10 | L8 and l4 | 74 |
| ☐ | L11 | e29$4 or glu-29$4 or glu29$4 | 5552 |
| ☐ | L12 | L11 same adjuvant$ | 8 |
| ☐ | L13 | L11 same (pea or exotoxin or exo-toxin or pseudomonas or holotoxin or holo-toxin or enterotoxin or toxin or cytotoxin) | 22 |
| ☐ | L14 | L13 not l12 | 17 |

END OF SEARCH HISTORY

L12: Entry 2 of 8 File: PGPB Aug 12, 2004

DOCUMENT-IDENTIFIER: US 20040157241 A1
TITLE: Haemophilus adherence and penetration proteins

Detail Description Paragraph:
[0100] Cholera toxin (CT) is well known as a potent mucosal adjuvant but is highly toxic to humans (Snider, D. P., 1995, Crit. Rev. Immunol 15:317-48). CT-E29H is a mutant form of CT that contains a histidine in place of a glutamic acid at residue 29 in the enzymatic A subunit. This mutant lacks enzymatic activity and has <1% of the cellular toxicity of native cholera toxin but remains fully active as an adjuvant, suggesting considerable utility in humans (Tebbey et al. 2000, Vaccine 18:2723-34). Accordingly, in a preferred embodiment the invention provides a composition comprising a HAP protein of the invention and cholera toxin CT-E29H. In addition the invention provides a method of improving immunization by administering an immunogenic protein of the invention and an adjuvant. In a preferred embodiment the adjuvant is CT-E29H.

Detail Description Paragraph:
[0156] Intranasal immunization of mice. Groups of ten, 6-week old, female Balb/c mice were immunized intranasally with Hap.sub.s purified from either strain P860295 or strain N187. Hap.sub.s was diluted in Dulbecco's PBS (D-PBS) to a final concentration of 5 or 15 .mu.g/40 .mu.l, with or without 0.1 .mu.g CT-E29H (a mutant cholera toxin used as an adjuvant) (Tebbey, et al., 2000, Vaccine 18:2723-34). Control mice received D-PBS alone or D-PBS with 0.1 .mu.g CT-E29H, again in 40 .mu.l volumes.

Detail Description Table CWU:
3TABLE 2 Systemic hmoral immune response in Balb/c mice after intranasal vaccination with Hap.sub.s admixed with or without CT-E29H Anti-Hap.sub.s Vaccine Route (40 .mu.l) Dose (.mu.g) Adjuvant ELISA (IgG) HAP IN 5 -- 1,604 HAP IN 15 -- 5,204 HAP IN 5 CT-E29H 4,653 HAP IN 15 CT-E29H 15,111 -- IN -- CT-E29H <500 1xPBS IN IN -- -- <500 Formalin Fixed IN -- <500 TN106.P2 6-week old female Balb/c mice were vaccinated week 0, 1, 3, & 5. Week 7 Sera - no antibody titers were detected in earlier bleeds.

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**Search Results** - Record(s) 1 through 8 of 8 returned.

☐ 1. 20040167068. 02 Sep 03. 26 Aug 04. Novel immunogenic compositions for the prevention and treatment of meningococcal disease. Zlotnick, Gary W., et al. 514/12; A61K038/17.

☐ 2. 20040157241. 15 Oct 03. 12 Aug 04. Haemophilus adherence and penetration proteins. St. Geme, Joseph W. III. 435/6; C12Q001/68.

☐ 3. 20040052816. 29 May 03. 18 Mar 04. Recombinant protective protein from streptococcus pneumoniale. Green, Bruce A., et al. 424/190.1; 435/252.3 435/320.1 435/69.3 530/350 536/23.7 C07K014/31 C07H021/04 A61K039/02 C12N001/21 C12N015/74.

☐ 4. 20030073166. 22 Feb 02. 17 Apr 03. Haemophilus adherence and penetration proteins. Geme, Joseph W. ST. III. 435/69.1; C12P021/06.

☐ 5. 6676948. 22 Feb 02; 13 Jan 04. Haemophilus adherence and penetration proteins. St. Geme, III; Joseph W.. 424/256.1; 424/185.1 424/190.1 530/350. A61K039/102.

☐ 6. WO 200253761A. Novel isolated 20 kDa Streptococcus pneumoniae surface associated pneumoprotective protein having ability to reduce colonization of pneumococcal bacteria, useful for eliciting immunity from otitis media, pneumonia. GREEN, B A, et al. A61K039/02 C07H021/04 C07K014/00 C07K014/31 C12N001/21 C12N015/63 C12N015/74 C12Q000/00.

☐ 7. WO 200228351A. Mucin binding proteins, useful in the induction of an immune response to, and in the diagnosis of, pneumococcal infections. GREEN, B A, et al. A61K000/00.

☐ 8. WO 200018434A. New mutant cholera holotoxin having a point mutation at amino acid position 29 of the A subunit useful as an adjuvant in an antigenic composition to enhance the immune response in a vertebrate host to a selected antigen from a pathogen. ELDRIDGE, J H, et al. A61K039/00 A61K039/002 A61K039/02 A61K039/095 A61K039/102 A61K039/106 A61K039/12 A61K039/15 A61K039/155 A61K039/245 A61K039/39 A61P037/04 C07K014/14 C07K014/22 C07K014/28 C07K014/285 C07K014:28 C12N001/15 C12N001/19 C12N001/21 C12N005/10 C12N015/09 C12N015/63 C12P021/02.

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| Terms | Documents |
|---|---|
| L11 same adjuvant$ | 8 |

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US 20040167068A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0167068 A1**

**Zlotnick et al.** (43) **Pub. Date: Aug. 26, 2004**

(54) **NOVEL IMMUNOGENIC COMPOSITIONS FOR THE PREVENTION AND TREATMENT OF MENINGOCOCCAL DISEASE**

(76) Inventors: **Gary W. Zlotnick**, New Windsor, NY (US); **Leah Diane Fletcher**, Geneseo, NY (US); **John Erwin Farley**, Rochester, NY (US); **Liesel A. Bernfield**, Pittsford, NY (US); **Robert J. Zagursky**, Victor, NY (US); **Benjamin J. Metcalf**, Rochester, NY (US)

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**INTELLECTUAL PROPERTY DEPARTMENT**
**1900 K STREET, N.W.**
**SUITE 1200**
**WASHINGTON, DC 20006-1109 (US)**

(21) Appl. No.: **10/652,870**

(22) Filed: **Sep. 2, 2003**

**Related U.S. Application Data**

(60) Provisional application No. 60/406,934, filed on Aug. 30, 2002.

**Publication Classification**

(51) **Int. Cl.**[7] ..... **A61K 38/17**

(52) **U.S. Cl.** ..... **514/12**

(57) **ABSTRACT**

The present invention relates to Neisseria ORF2086 proteins, crossreactive immunogenic proteins which can be isolated from neisserial strains or prepared recombinantly, including immunogenic portions thereof, biological equivalents thereof, antibodies that immunospecifically bind to the foregoing and nucleic acid sequences encoding each of the foregoing, as well as the use of same in immunogenic compositions that are effective against infection by *Neisseria meningitidis* serogroup B.

# WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, September 29, 2004

| Hide? | Set Name | Query | Hit Count |
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| | | *DB=USPT; PLUR=YES; OP=AND* | |
| ☐ | L1 | E47 or e-47 or glu47 or glu-47 or e47$1 or e-47$1 or glu47$3 or glu-47$3 | 488 |
| ☐ | L2 | L1 same (adjuvant$ or $toxin or ctx or lt or hlt or cta or cholera or vibrio or enterotoxin or entero-toxin or toxin) | 5 |

END OF SEARCH HISTORY

PileUp

MSF: 258 Type: P Check: 2315 ..

Name: sp|P01555|CHTA_VIBCH oo Len: 258 Check: 6104 Weight: 0.100
Name: tr|Q8VLI6 oo Len: 258 Check: 5874 Weight: 0.100
Name: tr|Q8L356 oo Len: 258 Check: 5942 Weight: 0.100
Name: sp|P06717|ELAP_ECOLI oo Len: 258 Check: 8172 Weight: 0.100
Name: tr|O66280 oo Len: 258 Check: 7907 Weight: 0.100
Name: sp|P43530|ELAH_ECOLI oo Len: 258 Check: 8316 Weight: 0.100

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tr|Q8L356                 MVKIIFVFFI FLSSFSYAND DKLYRADSRP PDEIKQSGGL MPRGQSEYFD
sp|P06717|ELAP_ECOLI      MKNITFIFFI LLASPLYANG DRLYRADSRP PDEIKRSGGL MPRGHNEYFD
tr|O66280                 MKNITFIFFI LLASPLYANG DKLYRADSRP PDEIKRSGGL MPRGHNEYFD
sp|P43530|ELAH_ECOLI      MKNITFIFFI LLASPLYANG DKLYRADSRP PDEIKRSGGL MPRGHNEYFD

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tr|Q8L356                 RGTQMNINLY DHARGTQTGF VRHDDGYVST SISLRSAHLV GQTILSGHST
sp|P06717|ELAP_ECOLI      RGTQMNINLY DHARGTQTGF VRYDDGYVST SLSLRSAHLA GQSILSGYST
tr|O66280                 RGTQMNINLY DHARGTQTGF VRYDDGYVST SLSLRSAHLA GQSILSGYST
sp|P43530|ELAH_ECOLI      RGTQMNINLY DHARGTQTGF VRYDDGYVST SLSLRSAHLA GQSILSGYST

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tr|Q8VLI6                 YYIYVIATAP NMFNVNDVLG AYSPHPDEQE VSALGGIPYS QIYGWYRVHF
tr|Q8L356                 YYIYVIATAP NMFNVNDVLG AYSPHPDEQE VSALGGIPYS QIYGWYRVHF
sp|P06717|ELAP_ECOLI      YYIYVIATAP NMFNVNDVLG VYSPHPYEQE VSALGGIPYS QIYGWYRVNF
tr|O66280                 YYIYVIATAP NMFNVNDVLG VYSPHPYEQE VSALGGIPYS QIYGWYRVNF
sp|P43530|ELAH_ECOLI      YYIYVIATAP NMFNVNDVLG VYSPHPYEQE VSALGGIPYS QIYGWYRVNF

sp|P01555|CHTA_VIBCH      GVLDEQLHRN RGYRDRYYSN LDIAPAADGY GLAGFPPEHR AWREEPWIHH
tr|Q8VLI6                 GVLDEQLHRN RGYRDRYYSN LDIAPAADGY GLAGFPPEHR AWREEPWIHH
tr|Q8L356                 GVLDEQLHRN RGYRDRYYSN LDIAPAADGY GLAGFPPEHR AWREEPWIHH
sp|P06717|ELAP_ECOLI      GVIDERLHRN REYRDRYYRN LNIAPAEDGY RLAGFPPDHQ AWREEPWIHH
tr|O66280                 GVIDERLHRN REYRDRYYRN LNIAPAEDGY RLAGFPPDHQ AWREEPWIHH
sp|P43530|ELAH_ECOLI      GVIDERLHRN REYRDRYYRN LNIAPAEDGY RLAGFPPDHQ AWREEPWIHH

sp|P01555|CHTA_VIBCH      APPGCGNAPR SSMSNTCDEK TQSLGVKFLD EYQSKVKRQI FSGYQSDIDT
tr|Q8VLI6                 APPGCGNAPR SSMSNTCDEK TQSLGVKFLD EYQSKVKRQI FSGYQSDIDT
tr|Q8L356                 APPGCGNAPR SSMSNTCDEK TQSLGVKFLD EYQSKVKRQI FSGYQSDIDT
sp|P06717|ELAP_ECOLI      APQGCGNSSR TITGDTCNEE TQNLSTIYLR EYQSKVKRQI FSDYQSEVDI
tr|O66280                 APQGCGNSSR TITDDTCNEE TQNLSTIYLR KYQSKVKRQI FSDYQSEVDI
sp|P43530|ELAH_ECOLI      APQGCGNSSR TITGDTCNEE TQNLSTIYLR KYQSKVKRQI FSDYQSEVDI

sp|P01555|CHTA_VIBCH      HNRIKDEL
tr|Q8VLI6                 HNRIKDEL
tr|Q8L356                 HNRIEDEL
```

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sp|P06717|ELAP_ECOLI        YNRIRDEL
tr|O66280                   YNRIRDEL
sp|P43530|ELAH_ECOLI        YNRIRNEL
```

DIALOG(R)File 155:MEDLINE(R)
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10393094 PMID: 7723660

**Expression and mutagenesis of recombinant cholera toxin A subunit.**

Vadheim K L; Singh Y; Keith J M

Laboratory of Microbial Ecology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892, USA.

Microbial pathogenesis (ENGLAND) Nov 1994, 17 (5) p339-46, ISSN 0882-4010 Journal Code: 8606191

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

ADP-ribosylating protein exotoxins from Vibrio cholerae (CT) and Escherichia coli (LT-I) share two short regions of sequence similarity with Bordetella pertussis toxin (PT). Previous studies have indicated that substitution of arginine for lysine 7 within the first region of CT drastically decreases ADP ribosyltransferase activity. We have more closely defined the role of other amino acids in this region by generating modified proteins in which arginine 7 was replaced with lysine (R7K), aspartate 9 was replaced with arginine (D9R), glycine was substituted for proline 12 (P12G), amino acids 6 to 13 were **deleted** (delta **613** ) or the C-terminal KDEL sequence was changed to NEDL. The modified proteins R7K, D9R and delta **613** exhibited undetectable ADP ribosyltransferase activity. Comparison of the tryptic digest of R7K with native CT suggested that changes in protein conformation may be responsible for the loss of ADP-ribosylation activity.

Tags: Support, Non-U.S. Gov't

Descriptors: **Cholera Toxin** --genetics--GE; Adenosine Diphosphate Ribose --metabolism--ME; Amino Acid Sequence; Base Sequence; Cholera Toxin --biosynthesis--BI; Cholera Toxin--metabolism--ME; DNA Mutational Analysis ; Molecular Sequence Data; Recombinant Proteins--biosynthesis--BI; Structure-Activity Relationship

CAS Registry No.: 0 (Recombinant Proteins); 20762-30-5 (Adenosine Diphosphate Ribose); 9012-63-9 (Cholera Toxin)

Record Date Created: 19950523

Record Date Completed: 19950523

**27/9/30 (Item 30 from file: 155)**

DIALOG(R)File 155:MEDLINE(R)
(c) format only 2004 The Dialog Corp. All rts. reserv.

10147805 PMID: 8039872

**Construction and characterization of recombinant Vibrio cholerae strains producing inactive cholera toxin analogs.**

Hase C C; Thai L S; Boesman-Finkelstein M; Mar V L; Burnette W N; Kaslow H R; Stevens L A; Moss J; Finkelstein R A

Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia 65212.

Infection and immunity (UNITED STATES) Aug 1994, 62 (8) p3051-7, ISSN 0019-9567 Journal Code: 0246127

Contract/Grant No.: AI17312; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The catalytic A subunit of cholera toxin (CT-A) is capable of ADP-ribosylating the guanine nucleotide-binding protein, which regulates cell adenylyl cyclase, leading to the life-threatening diarrhea of cholera. Amino acids involved in the enzymatic activity of CT-A have previously been identified. By means of site-directed mutagenesis, an analog of the CT-A subunit gene was created with codon substitutions for both Arg-7 and **Glu** -112, each of which has been shown to produce subunits lacking ADP-ribosyltransferase activity. The mutated gene fragment was exchanged

12750874 PMID: 7672106

**Identification of Glu173 as the critical amino acid residue for the ADP-ribosyltransferase activity of Clostridium botulinum C3 exoenzyme.**

Saito Y; Nemoto Y; Ishizaki T; Watanabe N; Morii N; Narumiya S
Department of Pharmacology, Kyoto University Faculty of Medicine, Japan.

FEBS letters (NETHERLANDS) Sep 4 1995, 371 (2) p105-9, ISSN 0014-5793 Journal Code: 0155157
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Clostridium botulinum C3 exoenzyme specifically ADP-ribosylates rho-p21 in eukaryotic cells. Trp18 and Glu173 of this enzyme were **substituted** with other amino acids via site-directed **mutagenesis** . All **substitutions** at Glu173 caused a significant reduction in affinity for NAD and diminished ADP-ribosyltransferase activity. On the other hand, the activity of enzymes with the **substitution** at Trp18 remained intact. Swiss 3T3 cells treated with the enzyme with the Trp18 **substitution** showed the typical morphologic changes of the C3 exoenzyme phenotype. In contrast, no changes were found in cells incubated with the Glu173- **substituted** enzyme. These results indicate that the Glu173 residue of the C3 exoenzyme plays a key role in interacting with NAD and in expression of ADP-ribosyltransferase activity, which is essential for the phenotypic change by C3 exoenzyme treatment.

Tags: Support, Non-U.S. Gov't
Descriptors: *ADP Ribose Transferases--chemistry--CH; *ADP Ribose Transferases--metabolism--ME; *Botulinum Toxins; *Glutamic Acid; *Poly(ADP-ribose) Polymerases--metabolism--ME; 3T3 Cells; **ADP Ribose Transferases** --genetics--GE; Animals; Base Sequence; Binding Sites; Mice; Molecular Sequence Data; **Mutagenesis** , Site-Directed; NAD--metabolism--ME; Structure-Activity Relationship; Tryptophan
CAS Registry No.: 0 (Botulinum Toxins); 53-84-9 (NAD); 56-86-0 (Glutamic Acid); 73-22-3 (Tryptophan)
Enzyme No.: EC 2.4.2.- (ADP Ribose Transferases); EC 2.4.2.- (exoenzyme C3, Clostridium botulinum); EC 2.4.2.30 (Poly(ADP-ribose) Polymerases)
Record Date Created: 19951016
Record Date Completed: 19951016

CLUSTAL W (1.74) multiple sequence alignment

```
sp|P01555|CHTA_VIBCH      MVKIIFVFFIFLSSFSYANDDKLYRADSRPPDEIKQSGGLMPRGQSEYFDRGTQMNIN
tr|Q8VLI6                 MVKIIFVFFIFLSSFSYANDDKLYRADSRPPDEIKQSGGLMPRGQNEYFDRGTQMNIN
tr|Q8L356                 MVKIIFVFFIFLSSFSYANDDKLYRADSRPPDEIKQSGGLMPRGQSEYFDRGTQMNIN
sp|P06717|ELAP_ECOLI      MKNITFIFFILLASPLYANGDRLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNIN
tr|O66280                 MKNITFIFFILLASPLYANGDKLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNIN
sp|P43530|ELAH_ECOLI      MKNITFIFFILLASPLYANGDKLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNIN
                          * :* *:***:*:*  ***.*:**************:********:.************

sp|P01555|CHTA_VIBCH      DHARGTQTGFVRHDDGYVSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVNDV
tr|Q8VLI6                 DHARGTQTGFVRHDDGYVSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVNDV
tr|Q8L356                 DHARGTQTGFVRHDDGYVSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVNDV
sp|P06717|ELAP_ECOLI      DHARGTQTGFVRYDDGYVSTSLSLRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDV
tr|O66280                 DHARGTQTGFVRYDDGYVSTSLSLRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDV
sp|P43530|ELAH_ECOLI      DHARGTQTGFVRYDDGYVSTSLSLRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDV
                          ************:********:*******.**:****:********************

sp|P01555|CHTA_VIBCH      AYSPHPDEQEVSALGGIPYSQIYGWYRVHFGVLDEQLHRNRGYRDRYYSNLDIAPAAD
tr|Q8VLI6                 AYSPHPDEQEVSALGGIPYSQIYGWYRVHFGVLDEQLHRNRGYRDRYYSNLDIAPAAD
tr|Q8L356                 AYSPHPDEQEVSALGGIPYSQIYGWYRVHFGVLDEQLHRNRGYRDRYYSNLDIAPAAD
sp|P06717|ELAP_ECOLI      VYSPHPYEQEVSALGGIPYSQIYGWYRVNFGVIDERLHRNREYRDRYYRNLNIAPAED
tr|O66280                 VYSPHPYEQEVSALGGIPYSQIYGWYRVNFGVIDERLHRNREYRDRYYRNLNIAPAED
sp|P43530|ELAH_ECOLI      VYSPHPYEQEVSALGGIPYSQIYGWYRVNFGVIDERLHRNREYRDRYYRNLNIAPAED
                          .***** ********************:***:**:***** ****** **:**** *

sp|P01555|CHTA_VIBCH      GLAGFPPEHRAWREEPWIHHAPPGCGNAPRSSMSNTCDEKTQSLGVKFLDEYQSKVKR
tr|Q8VLI6                 GLAGFPPEHRAWREEPWIHHAPPGCGNAPRSSMSNTCDEKTQSLGVKFLDEYQSKVKR
tr|Q8L356                 GLAGFPPEHRAWREEPWIHHAPPGCGNAPRSSMSNTCDEKTQSLGVKFLDEYQSKVKR
sp|P06717|ELAP_ECOLI      RLAGFPPDHQAWREEPWIHHAPQGCGNSSRTITGDTCNEETQNLSTIYLREYQSKVKR
tr|O66280                 RLAGFPPDHQAWREEPWIHHAPQGCGNSSRTITDDTCNEETQNLSTIYLRKYQSKVKR
sp|P43530|ELAH_ECOLI      RLAGFPPDHQAWREEPWIHHAPQGCGNSSRTITGDTCNEETQNLSTIYLRKYQSKVKR
                           ******:*:************ ****:.*:  .:**:*:**.*.. :* :********

sp|P01555|CHTA_VIBCH      FSGYQSDIDTHNRIKDEL
tr|Q8VLI6                 FSGYQSDIDTHNRIKDEL
tr|Q8L356                 FSGYQSDIDTHNRIEDEL
sp|P06717|ELAP_ECOLI      FSDYQSEVDIYNRIRDEL
tr|O66280                 FSDYQSEVDIYNRIRDEL
sp|P43530|ELAH_ECOLI      FSDYQSEVDIYNRIRNEL
                          **.***::* :***.:**
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# WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, September 29, 2004

| Hide? | Set Name | Query | Hit Count |
|---|---|---|---|
| | *DB=USPT; PLUR=YES; OP=AND* | | |
| ☐ | L1 | position.clm. same 29.clm. | 12569 |
| ☐ | L2 | L1 and adjuvant$.clm. | 10 |
| ☐ | L3 | L1 and adjuvant$.ti,ab. | 0 |
| ☐ | L4 | ribosylat$.ti,ab,clm.. or adpribosy$.ti,ab,clm. | 46 |
| ☐ | L5 | L4 and 29 | 30 |
| ☐ | L6 | L5 not l2 | 30 |
| ☐ | L7 | l1 and l4 | 0 |
| ☐ | L8 | position near30 29 | 93692 |
| ☐ | L9 | L8 same (ribosylat$ or adp or adjuvant$ or cholera or hlt or ribosyltransferase or pertuss$) | 6 |
| ☐ | L10 | l8 and $toxin | 390 |
| ☐ | L11 | L10 and vaccin$ | 168 |
| ☐ | L12 | l8 same $toxin | 24 |

END OF SEARCH HISTORY

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L2: Entry 3 of 10 File: USPT Aug 26, 2003

DOCUMENT-IDENTIFIER: US 6610300 B1
TITLE: Clostridium perfringens vaccine

CLAIMS:

5. The derivative of Clostridium perfringens .beta.-toxin or an immunogenic fragment fragment thereof according to claim 1, wherein the mutation is located in the .beta.-toxin of SEQ ID NO: 29 at position 62, 182, 197 or in one of the regions between amino acid numbers 80-103, 145-147, 281-291, 295-299 or downstream of amino acid position 292.

8. The immunogenic composition according to claim 7, further comprising an adjuvant.

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L2: Entry 3 of 10 File: USPT Aug 26, 2003

US-PAT-NO: 6610300
DOCUMENT-IDENTIFIER: US 6610300 B1

TITLE: Clostridium perfringens vaccine

DATE-ISSUED: August 26, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Segers; Ruud Philip Antoon Maria | Boxmeer | | | NL |
| Waterfield; Nicolas Robin | Cambridge | | | GB |
| Frandsen; Peer Lyng | Holte | | | DK |
| Wells; Jeremy Mark | Cambridge | | | GB |

ASSIGNEE-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY | TYPE CODE |
|---|---|---|---|---|---|
| Akzo Nobel N.V. | Arnhem | | | NL | 03 |

APPL-NO: 09/ 100703 [PALM]
DATE FILED: June 19, 1998

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---|---|---|
| EP | 97201888 | June 20, 1997 |

INT-CL: [07] A61 K 39/00, A61 K 39/38, A61 K 39/02, A61 K 39/08, C07 K 1/00

US-CL-ISSUED: 424/184.1; 424/234.1, 424/236.1, 424/239.1, 424/247.1, 530/350
US-CL-CURRENT: 424/184.1; 424/234.1, 424/236.1, 424/239.1, 424/247.1, 530/350

FIELD-OF-SEARCH: 530/350, 424/184.1, 424/234.1, 424/236.1, 424/239.1, 424/247.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS



| | PAT-NO | ISSUE-DATE | PATENTEE-NAME | US-CL |
|---|---|---|---|---|
| ☐ | 5817317 | October 1998 | Titball et al. | |

FOREIGN PATENT DOCUMENTS

| FOREIGN-PAT-NO | PUBN-DATE | COUNTRY | US-CL |
|---|---|---|---|
| 958574 | May 1964 | GB | |
| 2030451 | April 1980 | GB | |
| WO 9323543 | November 1993 | WO | |
| WO 9717521 | June 1995 | WO | |
| WO 97/34001 | September 1997 | WO | |

OTHER PUBLICATIONS

Hunter et al., Infection and Immunity, 61:9:3958-3965, 1993.
Sakurai and Duncan, Infection and Immunity, 18:3:741-745, 1977.

ART-UNIT: 1645

PRIMARY-EXAMINER: Navarro; Mark

ATTY-AGENT-FIRM: Blackstone; William M.

ABSTRACT:

The present invention relates to detoxified immunogenic derivatives of Clostridium perfringens .beta.-toxin or an immunogenic fragment thereof that have as a characteristic that they carry a mutation in the .beta.-toxin amino acid sequence, not found in the wild-type .beta.-toxin amino acid sequence. The invention also relates to genes encoding such .beta.-toxins, as well as to expression systems expressing such .beta.-toxins. Moreover, the invention relates to bacterial expression systems expressing a native .beta.-toxin. Finally, the invention relates to vaccines based upon detoxified immunogenic derivatives of Clostridium perfringens .beta.-toxin, and methods for the preparation of such vaccines.

14 Claims, 60 Drawing figures

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L6: Entry 6 of 30 File: USPT Aug 27, 2002

DOCUMENT-IDENTIFIER: US 6440423 B1
TITLE: Mutant enterotoxin effective as a non-toxic oral adjuvant

Brief Summary Text (1):
TABLE OF CONTENTS 1. FIELD OF THE INVENTION 2. BACKGROUND OF THE INVENTION 3. SUMMARY OF THE INVENTION 4. BRIEF DESCRIPTION OF THE FIGURES 5. DETAILED DESCRIPTION OF THE INVENTION 5.1 PRODUCTION OF mLT 5.2 MODE OF ADMINISTRATION OF mLT AND UNRELATED ANTIGEN 6. EXAMPLES 6.1 CONSTRUCTION OF mLT 6.2 EFFECT OF mLT ON Y-1 ADRENAL CELLS 6.3 ADP-RIBOSYLATING ENZYMATIC ACTIVITY OF mLT 6.4 ENTEROTOXIC ACTIVITY OF mLT 29 6.5 ADJUVANT ACTIVITY OF mLT 6.5.1 SERUM IgG ANTI-OVA 6.5.2 MUCOSAL sIgA ANTI-OVA 6.5.3 SERUM IgG ANTI-LT 6.5.4 MUCOSAL sIgA ANTI-LT 7. DEPOSIT OF MICROORGANISMS

Brief Summary Text (8):
A number of strategies have been developed for oral immunization, including the use of attenuated mutants of bacteria (i.e., Salmonella spp.) as carriers of heterologous antigens [Cardenas and Clements, 1992, Clin. Microbiol. Rev. 5:328-342; Clements et al., 1992, In: Recombinant DNA Vaccines: Rationale and Strategy, Isaacson (ed.), Marcel Decker, New York. p.p. 293-321; Clements and Cardenas, 1990, Res. Microbiol. 141:981-993; Clements and El-Morshidy, 1984, Infect. Immun. 46:564-569], encapsulation of antigens into microspheres composed of poly-DL-lactide-glycolide (PGL), protein-like polymers-proteinoids [Sanitago et al., 1993, Pharmaceutical Research 10:1243-1247], gelatin capsules, different formulations of liposomes [Alving et al., 1986, Vaccine 4:166-172; Garcon and Six, 1993, J. Immunol. 146:3697-3702; Gould-Fogerite and Mannino, 1993, In: Liposome Technology 2nd Edition. Vol. III, Gregoriadis (ed.)], adsorption onto nanoparticles, use of lipophilic immune stimulating complexes (ISCOMS) [Mowat and Donachie, 1991, Immunology Today 12:383-385], and addition of bacterial products with known adjuvant properties [Clements et al., 1988, Vaccine 6:269-277; Elson, 1989, Immunology Today 146:29-33; Lycke and Holmgren, 1986, Immunology 59:301-308; Lycke et al., 1992, Eur. J. Immunol. 22:2277-2281]. The two bacterial products with the greatest potential to function as oral adjuvants are cholera toxin (CT), produced by various strains of V. cholerae, and the heat-labile enterotoxin (LT) produced by some enterotoxigenic strains of Escherichia coli. Although LT and CT have many features in common, these are clearly distinct molecules with biochemical and immunologic differences which make them unique.

Brief Summary Text (11):
In addition to being a potent oral immunogen, CT has a number of other reported immunologic properties. As indicated above, Elson and Ealding [Elson and Ealding, 1984, J. Immunol. 133: 2892-2897] observed that orally administered CT does not induce tolerance against itself. Moreover, simultaneous oral administration of CT with a soluble protein antigen, keyhole limpet hemocyanin (KLH), resulted in the development of secretory IgA responses against both CT and KLH and also abrogated the induction of oral tolerance against KLH. These findings were subsequently confirmed and extended by Lycke and Holmgren [Lycke and Holmgren, 1986, Immunology 59:301-308]. The confusion arises when one attempts to define the role of the A and B subunits of CT with respect to the adjuvant properties of the molecule. The following observations, as summarized by Elson [Elson, 1989, Immunology Today

146:29-33], are the basis for that confusion: CT does not induce oral tolerance against itself [Elson and Ealding, 1984, J. Immunol. 133: 2892-2897]. CT-B does not induce oral tolerance against itself [Elson and Ealding, 1984, J. Immunol. 133: 2892-2897]. CT can prevent the induction of tolerance against other antigens with which it is simultaneously delivered and also serve as an adjuvant for those antigens [Elson and Ealding, 1984, J. Immunol. 133: 2892-2897; Lycke and Holmgren, 1986, Immunology 59:301-308]. CT can act as and adjuvant for CT-B [Elson and Ealding, 1984, J. Immunol. 133: 2892-2897]. Heat aggregated CT has little toxicity but is a potent oral immunogen [Pierce et al., 1983, Infect. Immun. 40: 1112-1118]. CT-B can serve as an immunologic "carrier" in a traditional hapten-carrier configuration [Cebra et al., 1986, In: Vaccines 86, Brown et al. (ed.), Cold Spring Harbor Laboratory, New York. p.p. 129-133; McKenzie and Halsey, 1984, J. Immunol. 133: 1818-1824].

Other Reference Publication (27):
Elson, 1989, "Cholera toxin and its subunits as potential oral adjuvants", Curr. Topics Microbiol. Immunol. 146:29-33.

Other Reference Publication (47):
Clements et al., 1980, "Properties of homogenous heat-labile enterotoxin from Escherichia coli", Infect. Immun. 29:91-97.

CLAIMS:

1. A vaccine preparation comprising an antigen in combination with a composition comprising a mutant E. coli heat-labile enterotoxin holotoxin, in which arginine at amino acid position 192 is replaced with glycine, which holotoxin is substantially less toxic than native E. coli heat-labile enterotoxin holotoxin as measured in the Y-1 adrenal cell assay and which has immunologic adjuvant activity but lacks ADP-ribosylating enzymatic activity as measured by the NAD-Agmatine ADP-ribosyltransferase assay.

7. A composition comprising (a) a vaccine selected from the group consisting of influenza vaccine, pertussis vaccine, diphtheria and tetanus toxoid combined with pertussis vaccine, hepatitis A vaccine, hepatitis B vaccine, hepatitis C vaccine, hepatitis E vaccine, Japanese encephalitis vaccine, herpes vaccine, measles vaccine, rubella vaccine, mumps vaccine, mixed vaccine of measles, mumps and rubella, papillomavirus vaccine, parvovirus vaccine, respiratory syncytial virus vaccine, Lyme disease vaccine, polio vaccine, malaria vaccine, varicella vaccine, gonorrhea vaccine, schistosomiasis vaccine, rota vaccine, Campylobacter vaccine, cholera vaccine, enteropathogenic E. coli vaccine, enterotoxic E. coli vaccine, mycoplasma vaccine, pneumococcal vaccine, and meningococcal vaccine, and (b) a composition comprising a mutant E. coli heat-labile enterotoxin holotoxin, in which arginine at amino acid position 192 is replaced with glycine, which holotoxin is substantially less toxic than native E. coli heat-labile enterotoxin holotoxin as measured in the Y-1 adrenal cell assay and which has immunologic adjuvant activity but lacks ADP-ribosylating enzymatic activity as measured by the NAD-Agmatine ADP-ribosyltransferase assay.

8. A kit useful in producing a protective immune response in a host to a pathogen comprising two components: (a) an effective amount of a protective antigen of a bacterial, viral or fungal pathogen, and (b) an adjuvant effective amount of a mutant E. coli heat-labile enterotoxin holotoxin, in which arginine at amino acid position 192 is replaced with glycine, and which has immunologic adjuvant activity but lacks ADP-ribosylating enzymatic activity as measured by the NAD-Agmatine ADP-ribosyltransferase assay, wherein both said components are in an orally acceptable carrier and said components may be administered either after having been mixed together or separately.

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US006716431B1

(12) **United States Patent**
**Tian et al.**

(10) **Patent No.: US 6,716,431 B1**
(45) **Date of Patent: Apr. 6, 2004**

(54) **DIFFERENTIAL CYTOTOXICITY OF ALTERNATIVE FORMS OF ROTAVIRUS NONSTRUCTURAL PROTEIN 4**

(75) Inventors: **Peng Tian**, Monroe, NY (US); **Timothy J. Zamb**, Nyack, NY (US); **Stephen A. Udem**, New York, NY (US)

(73) Assignee: **Wyeth Holdings Corporation**, Madison, NJ (US)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/048,540**

(22) PCT Filed: **May 28, 1999**

(86) PCT No.: **PCT/US99/11872**

§ 371 (c)(1),
(2), (4) Date: **Dec. 7, 2001**

(87) PCT Pub. No.: **WO99/61621**

PCT Pub. Date: **Dec. 2, 1999**

**Related U.S. Application Data**

(60) Provisional application No. 60/087,320, filed on May 29, 1998.

(51) **Int. Cl.**[7] ..... **A61K 39/15**; C12N 7/01; C12N 15/46; C12N 15/63

(52) **U.S. Cl.** ..... **424/215.1**; 435/235.1; 435/236; 435/320.1; 435/69.3; 536/23.72

(58) **Field of Search** ..... 435/235.1, 236, 435/252.3, 325, 69.1, 69.3; 424/215.1; 536/23.72

(56) **References Cited**

PUBLICATIONS

Ciarlet et al. Archives of Virology 145:371–383, 2000.*
Cunliffe et al. Journal of Medical Virology 53:41–50, Sep. 1997.*
Horie et al. Archives of Virology 142:1865–1872, Sep. 1997.*

* cited by examiner

*Primary Examiner*—Mary Mosher
(74) *Attorney, Agent, or Firm*—J. Darrell Fontenot

(57) **ABSTRACT**

The nonstructural protein 4 (NSP4) in the SA11 ATCC rotavirus strain has a histidine at amino acid position 47. This substituted form is more cytotoxic than the NSP4 of the Australia rotavirus strain, which has an asparagine at amino acid position 47. The histidine at amino acid position 47 is mutagenized to another amino acid to produce an alternative form of NSP4 which has reduced toxicity, while retaining its antigenicity and immunogenicity. NSP4 having a glutamic acid at amino acid position 48 is more cytotoxic than NSP4 having a lysine at amino acid position 48. The lysine at amino acid position 48 is mutagenized to another amino acid other than glutamic acid to produce an alternative form of NSP4 which has reduced toxicity, while retaining its antigenicity and immunogenicity.

**17 Claims, 6 Drawing Sheets**

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L9: Entry 1 of 6 File: USPT Apr 6, 2004

DOCUMENT-IDENTIFIER: US 6716431 B1
TITLE: Differential cytotoxicity of alternative forms of rotavirus nonstructural protein 4

Detailed Description Text (28):
Antigenic compositions containing an alternative form of NSP4 protein may be mixed with immunologically acceptable diluents or carriers in a conventional manner to prepare injectable liquid Is solutions or suspensions. The level of antibodies elicited by the antigenic compositions may be improved by using certain adjuvants such as Stimulon.TM. QS-21 (Aquila Biopharmaceuticals, Inc., Framingham, Mass.), MPL.TM. (3-O-deacylated monophosphoryl lipid A; RIBI ImmunoChem Research, Inc., Hamilton, Mont.), aluminum phosphate, aluminum hydroxide, IL-12 (Genetics Institute, Cambridge, Mass.) and cholera toxin (either in a wild-type or mutant form, for example wherein the glutamic acid at amino acid position 29 is replaced by another amino acid, preferably a histidine, in accordance with U.S. Provisional Patent Application No. 60/102,430).

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L12: Entry 21 of 24 File: USPT Nov 19, 1991

DOCUMENT-IDENTIFIER: US 5066593 A

**** See image for Certificate of Correction ****

TITLE: Synthetic peptide-based anti-rabies compositions and methods

Brief Summary Text (24):

Numerous curaremimetic, snake-venom neurotoxins, similar to alph-a-bungarotoxin, are known which bind with high affinity at the ACh binding site of the AChR at neuromuscular junctions. The sequences of more than 60 of these neurotoxins are known. Studies of these neurotoxins, involving three-dimensional structural determinations, chemical modifications, and comparisons of sequences, have led to identification of four highly conserved amino acids which interact to form and stabilize a structure, which is similar to that of ACh and is thought to be involved in the binding of the neurotoxins to the active-site (i.e., the ACh binding-site) of the AChR. According to the amino acid numbering system based on the alignment of neurotoxin sequences by Karlsson (Handbook of Experimental Pharmacology 52, 159-212(1979), these four residues are tryptophan at position 29, aspartate at position 31, arginine at position 37 and glycine at position 38. The tryptophan at position 29 is thought to stabilize an ion-pair formed between the carboxylate group of aspartate-31 and the guanidinium group of arginine-37. It is this ion-pair which is thought to stereochemically mimic acetylcholine (Tsernoglou et al., Mol. Pharmacol. 14, 710(1978)). Modification of the tryptophan-29 results in a loss of toxic activity of no more than about 50% (Ryden et al., Int'l. Jour. Peptide Protein Res. 5, 261-273(1973)).

Previous Doc Next Doc Go to Doc#

CLUSTAL W (1.74) multiple sequence alignment

```
sp|P01555|CHTA_VIBCH    ----------------------------------------------------------MV
tr|Q7DCA1               MHIQSLQQSPSFAVELHQAASGRLGQIEARQVATPSEAQQLAQRQDAPKGEGLLARLG
                                                                                  :

sp|P01555|CHTA_VIBCH    IFVFFIFLSSFSYANDDKLYRADSRP-PDEIKQSGGLMPRGQSEYFDRGTQMNINLYD
tr|Q7DCA1               LVRPFVAIMDWLGKLLGSHARTGPQPSQDAQPAVMSSAVVFKQMVLQQALPMTLKGLD
                        :.  *: : .:     ..  *:..:*  *      .     :.  :::.  *.::  *

sp|P01555|CHTA_VIBCH    RGTQT----GFVRHDDGYVSTSISLRSAHLVGQTILSGHSTYYIYVIATAPNMFNVN-
tr|Q7DCA1               SELATLTPEGLAREHSRLASGDGALRSLSTALAGIRAGSQVEESRIQAGRLLERSIGG
                            *    *:.*...  .* . :***   .   * :* ..    : *      .:.

sp|P01555|CHTA_VIBCH    LGAYSPHPDEQEVSALGGIPYSQIYGWYRVHFGVLDEQLHR-------NRGYRDRYYS
tr|Q7DCA1               LQQWGTTGGAASQLVLDASPELRREITDQLHQVMSEVALLRQAVESEVSRVSADKALA
                        *  :..  .  .  .*.. *  :     ::*  : :  * *       .*   *:  :

sp|P01555|CHTA_VIBCH    DIAPA-ADGYGLAGFPP-------EHRAWREEPWIHHAPPGCGNAPRSSMSNTCDEKT
tr|Q7DCA1               LVKRFGADAEKYLGRQPGGIHSDAEVMALGLYTGIHYADLNRALRQGQELDAGQKLID
                         :    **.    *  *       *  *    . **:*  . .    ..:.   .

sp|P01555|CHTA_VIBCH    LGVKFLDEYQSKVKRQIFSGYQSDIDTHNRIKDEL----------------------
tr|Q7DCA1               MSAAFEKSGQAEQVVKTFRGTRG-GDAFNAVEEGKVGHDDGYLSTSLNPGVARSFGQG
                        :.. * .. *::   : * * :.  *:.* :::

sp|P01555|CHTA_VIBCH    ------------------------------------------------------------
tr|Q7DCA1               STVFGRSGIDVSGISNYKNEKEILYNKETDMRVLLSASDEQGVTRRVLEEAALGEQSG

sp|P01555|CHTA_VIBCH    ---------------------------------
tr|Q7DCA1               QGLLDALDLASKPERSGEVQEQDVRLRMRGLDLA
```

PileUp

MSF: 454 Type: P Check: 5989 ..

Name: sp|P01555|CHTA_VIBCH oo Len: 454 Check: 3989 Weight: 0.100
Name: tr|Q7DCA1 oo Len: 454 Check: 2000 Weight: 0.100

//

```
sp|P01555|CHTA_VIBCH      .......... .......... .......... .......... ..........
tr|Q7DCA1                 MHIQSLQQSP SFAVELHQAA SGRLGQIEAR QVATPSEAQQ LAQRQDAPKG

sp|P01555|CHTA_VIBCH      ......MVKI IFVFFIFLSS FSYANDDKLY RADSRP.PDE IKQSGGLMPR
tr|Q7DCA1                 EGLLARLGAA LVRPFVAIMD WLGKLLGSHA RTGPQPSQDA QPAVMSSAVV

sp|P01555|CHTA_VIBCH      GQSEYFDRGT QMNINLYDHA RGTQT....G FVRHDDGYVS TSISLRSAHL
tr|Q7DCA1                 FKQMVLQQAL PMTLKGLDKA SELATLTPEG LAREHSRLAS GDGALRSLST

sp|P01555|CHTA_VIBCH      VGQTILSGHS TYYIYVIATA PNMFNVN.DV LGAYSPHPDE QEVSALGGIP
tr|Q7DCA1                 ALAGIRAGSQ VEESRIQAGR LLERSIGGIA LQQWGTTGGA ASQLVLDASP

sp|P01555|CHTA_VIBCH      YSQIYGWYRV HFGVLDEQLH R.......NR GYRDRYYSNL DIAPA.ADGY
tr|Q7DCA1                 ELRREITDQL HQVMSEVALL RQAVESEVSR VSADKALADG LVKRFGADAE

sp|P01555|CHTA_VIBCH      GLAGFPP... ....EHRAWR EEPWIHHAPP GCGNAPRSSM SNTCDEKTQS
tr|Q7DCA1                 KYLGRQPGGI HSDAEVMALG LYTGIHYADL NRALRQGQEL DAGQKLIDQG

sp|P01555|CHTA_VIBCH      LGVKFLDEYQ SKVKRQIFSG YQSDIDTHNR IKDEL..... ..........
tr|Q7DCA1                 MSAAFEKSGQ AEQVVKTFRG TRG.GDAFNA VEEGKVGHDD GYLSTSLNPG

sp|P01555|CHTA_VIBCH      .......... .......... .......... .......... ..........
tr|Q7DCA1                 VARSFGQGTI STVFGRSGID VSGISNYKNE KEILYNKETD MRVLLSASDE

sp|P01555|CHTA_VIBCH      .......... .......... .......... .......... ..........
tr|Q7DCA1                 QGVTRRVLEE AALGEQSGHS QGLLDALDLA SKPERSGEVQ EQDVRLRMRG

sp|P01555|CHTA_VIBCH      ....
tr|Q7DCA1                 LDLA
```

# Search in Swiss-Prot and TrEMBL for: adp toxin

## Swiss-Prot Release 44.6 of 27-Sep-2004
## TrEMBL Release 27.6 of 27-Sep-2004

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- Number of sequences found in Swiss-Prot$_{(8)}$ and TrEMBL$_{(56)}$: **64**
- Note that the selected sequences can be saved to a file to be later retrieved; to do so, go to the bottom of this page.
- For more directed searches, you can use the Sequence Retrieval System SRS.

---

### Search in Swiss-Prot: There are matches to 8 out of 159201 entries

AEXT_AERSA (**Q93Q17**)
ADP-ribosyltransferase toxin aexT (EC 2.4.2.-) (Exoenzyme T) (aexT protein). {GENE: Name=aexT} - Aeromonas salmonicida

CHTA_VIBCH (**P01555**)
Cholera enterotoxin, A chain precursor (NAD(+)--diphthamide ADP-ribosyltransferase) (EC 2.4.2.36) (Cholera enterotoxin A subunit) [Contains: Cholera enterotoxin chain-A1 (Cholera enterotoxin alpha chain); Cholera enterotoxin chain-A2 (Cholera enterotoxin gamma chain)]. {GENE: Name=ctxA; Synonyms=toxA; OrderedLocusNames=VC1457} - Vibrio cholerae

DTX_CORBE (**P00588**)
Diphtheria toxin precursor (DT) (NAD(+)--diphthamide ADP-ribosyltransferase) (EC 2.4.2.36). - Corynephage beta

DTX_COROM (**P00587**)
Diphtheria toxin precursor (DT) (NAD(+)--diphthamide ADP-ribosyltransferase) (EC 2.4.2.36). - Corynephage omega

TOX1_BORPE (**P04977**)
Pertussis toxin subunit 1 precursor (PTX S1) (Islet-activating protein S1) (IAP S1) (NAD-dependent ADP-ribosyltransferase) (EC 2.4.2.-). {GENE: Name=ptxA; OrderedLocusNames=BP3783} - Bordetella pertussis

TOXA_PSEAE (**P11439**)
Exotoxin A precursor (NAD-dependent ADP-ribosyltransferase) (EC 2.4.2.-). {GENE: Name=eta; OrderedLocusNames=PA1148} - Pseudomonas aeruginosa

TXA2_RADPA (**P01534**)
Neurotoxin II (Toxin RpII) (Sodium channel toxin II). - Radianthus paumotensis (Sea anemone) (Heteractis paumotensis)

TXA3_RADPA (**P08380**)
Neurotoxin III (Toxin RpIII) (Sodium channel toxin III). - Radianthus paumotensis (Sea anemone) (Heteractis paumotensis)

---

## Search in TrEMBL: There are matches to 56 out of 1400820 entries

O49163
NADPH HC toxin reductase {GENE:Name=hm1} - Zea mays (Maize)
O49164
NADPH HC toxin reductase {GENE:Name=hm1} - Zea mays (Maize)
O49165
NADPH HC toxin reductase {GENE:Name=hm1} - Zea mays (Maize)
O49166
NADPH HC toxin reductase {GENE:Name=hm1} - Zea mays (Maize)
O49167
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
P93188
NADPH-dependent HC-toxin reductase - Hordeum vulgare (Barley)
Q41867
NADPH HC-toxin reductase - Zea mays (Maize)
Q6BMF6
Similar to CA3723|CaDPH2 Candida albicans CaDPH2 Diphtheria toxin resistance protein {GENE:ORFNames=DEHA0F06336g} - Debaryomyces hansenii CBS767
Q6VY12
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea diploperennis (Diploperennial teosinte)
Q6VY16
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea diploperennis (Diploperennial teosinte)
Q6VY17
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea diploperennis (Diploperennial teosinte)
Q6VY19
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea perennis (Perennial teosinte)
Q6VY23
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea perennis (Perennial teosinte)
Q6VY25
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea perennis (Perennial teosinte)
Q6VY27
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea perennis (Perennial teosinte)
Q6VY29
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea perennis (Perennial teosinte)
Q6ZJE8
Putative NADPH HC toxin reductase {GENE:Name=OJ1579_C03.16} - Oryza sativa (japonica cultivar-group)
Q7WUH3
Binary ADP-ribosyltransferase CDT toxin {GENE:Name=cdt} - Clostridium difficile
Q7X6N6
Putative NADPH HC toxin reductase {GENE:Name=OJ2013_G04.105;

Synonyms=OJ1634_B10.127} - Oryza sativa (japonica cultivar-group)
Q7X7U5
Putative NADPH HC toxin reductase {GENE:Name=OJ2013_G04.104; Synonyms=OJ1634_B10.126} - Oryza sativa (japonica cultivar-group)
Q7XIG2
Putative NADPH HC toxin reductase {GENE:Name=OJ2013_G04.121; Synonyms=OSJNBb0018H10.1} - Oryza sativa (japonica cultivar-group)
Q8L3V4
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
Q8L3V5
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L4E1
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (Maize)
Q8L8C9
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8D0
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8D1
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8D2
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8D3
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8D4
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8D5
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
Q8L8D6
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
Q8L8D7
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
Q8L8D8
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
Q8L8D9
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
Q8L8E0
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
Q8L8E1
NADPH HC toxin reductase (Fragment) {GENE:Name=hm2} - Zea mays (Maize)
Q8L8E2
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8E3
Truncated NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp.

parviglumis) (Balsas teosinte)
Q8L8E4
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8E5
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8E6
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8E7
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8E8
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8E9
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8F0
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8F1
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (subsp. parviglumis) (Balsas teosinte)
Q8L8F2
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (Maize)
Q8L8F3
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (Maize)
Q8L8F4
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (Maize)
Q8L8F5
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (Maize)
Q8L8F6
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (Maize)
Q8L8F7
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (Maize)
Q8L8F8
NADPH HC toxin reductase (Fragment) {GENE:Name=hm1} - Zea mays (Maize)
Q9RM75
Clostridium difficile binary toxin A (Actin-specific adp-ribosyltransferase) (Fragment) {GENE:Name=cdtA} - Clostridium difficile
Q9RM76
Clostridium difficile binary toxin A (Actin-specific adp-ribosyltransferase) (Fragment) {GENE:Name=cdtA} - Clostridium difficile

---

New Search
in Swiss-Prot/TrEMBL by AC, ID, description,

gene name, organism
**Please do NOT use any boolean operators (and, or, etc.)**

---

If you would like to retrieve all the Swiss-Prot/TrEMBL entries contained in this list , you can enter a file name. These entries will then be saved to a file under this name in the directory `outgoing` of the ExPASy anonymous ftp server, from where you can download it. (Please note that this temporary file will only be kept for 1 week.)

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# NiceProt View of Swiss-Prot: P11439



*Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.*

**Entry information**

| | |
|---|---|
| Entry name | **TOXA_PSEAE** |
| Primary accession number | **P11439** |
| Secondary accession number | Q9I4I7 |
| Entered in Swiss-Prot in | Release 12, October 1989 |
| Sequence was last modified in | Release 40, October 2001 |
| Annotations were last modified in | Release 45, October 2004 |

**Name and origin of the protein**

| | |
|---|---|
| Protein name | **Exotoxin A [Precursor]** |
| Synonyms | **NAD-dependent ADP-ribosyltransferase**<br>**EC 2.4.2.-** |
| Gene name | **Name: eta**<br>OrderedLocusNames: PA1148 |
| From | Pseudomonas aeruginosa [TaxID: 287] |
| Taxonomy | Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas. |

**References**

[1] SEQUENCE FROM NUCLEIC ACID, AND SEQUENCE OF 26-53.
MEDLINE=84194063;PubMed=6201861 [NCBI, ExPASy, EBI, Israel, Japan]
Gray G.L., Smith D.H., Baldridge J.S., Harkins R.N., Vasil M.L., Chen E.Y., Heyneker H.L.;
"Cloning, nucleotide sequence, and expression in Escherichia coli of the exotoxin A structural gene of Pseudomonas aeruginosa.";
Proc. Natl. Acad. Sci. U.S.A. 81:2645-2649(1984).

[2] SEQUENCE FROM NUCLEIC ACID.
**STRAIN**=ATCC 15692 / PAO1;
DOI=10.1038/35023079;MEDLINE=20437337;PubMed=10984043 [NCBI, ExPASy, EBI, Israel, Japan]
Stover C.K., Pham X.-Q.T., Erwin A.L., Mizoguchi S.D., Warrener P., Hickey M.J., Brinkman F.S.L., Hufnagle W.O., Kowalik D.J., Lagrou M., Garber R.L., Goltry L., Tolentino E., Westbrock-Wadman S., Yuan Y., Brody L.L., Coulter S.N., Folger K.R., Kas A., Larbig K., Lim R.M., Smith

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Nature 406:959-964(2000).

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MEDLINE=87250491;PubMed=2885323 [NCBI, ExPASy, EBI, Israel, Japan]
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[6] DISULFIDE BOND.
DOI=10.1021/bi991308+;MEDLINE=20068844;PubMed=10600112 [NCBI, ExPASy, EBI, Israel, Japan]
McKee M.L., FitzGerald D.J.;
"Reduction of furin-nicked Pseudomonas exotoxin A: an unfolding story.";
Biochemistry 38:16507-16513(1999).

[7] X-RAY CRYSTALLOGRAPHY (3.0 ANGSTROMS) OF 424-638.
MEDLINE=96016159;PubMed=7568123 [NCBI, ExPASy, EBI, Israel, Japan]
Li M., Dyda F., Benhar I., Pastan I., Davies D.R.;
"The crystal structure of Pseudomonas aeruginosa exotoxin domain III with nicotinamide and AMP: conformational differences with the intact exotoxin.";
Proc. Natl. Acad. Sci. U.S.A. 92:9308-9312(1995).

[8] X-RAY CRYSTALLOGRAPHY (2.3 ANGSTROMS) OF 424-638.
DOI=10.1073/pnas.93.14.6902;MEDLINE=96293446;PubMed=8692916 [NCBI, ExPASy, EBI, Israel, Japan]
Li M., Dyda F., Benhar I., Pastan I., Davies D.R.;
"Crystal structure of the catalytic domain of Pseudomonas exotoxin A complexed with a nicotinamide adenine dinucleotide analog: implications for the activation process and for ADP ribosylation.";
Proc. Natl. Acad. Sci. U.S.A. 93:6902-6906(1996).

**Comments**

- ***FUNCTION***: This toxin is a NAD-dependent ADP-ribosyltransferase. It catalyzes the transfer of the ADP ribosyl moiety of oxidized NAD onto elongation factor 2 (EF-2) thus arresting protein synthesis.
- ***PTM***: The 8 cysteines participate in intrachain disulfide bonds.
- ***SIMILARITY***: REGIONAL SEQUENCE SIMILARITY AT THE ACTIVE SITE WITH DIPHTHERIA TOXIN (DT).

## Copyright



## Cross-references

| | |
|---|---|
| EMBL | K01397; AAB59097.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]<br>AE004544; AAG04537.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence] |
| PIR | A30347; A30347.<br>C83503; C83503. |
| PDB | 1AER; X-ray; A=425-634, B=425-625.[ExPASy / RCSB / EBI]<br>1DMA; X-ray; A/B=425-638. [ExPASy / RCSB / EBI]<br>1IKP; X-ray; A=26-638. [ExPASy / RCSB / EBI]<br>1IKQ; X-ray; A=26-638. [ExPASy / RCSB / EBI]<br>Detailed list of linked structures. |
| SWISS-3DIMAGE | P11439. |
| CMR | P11439; PA1148. |
| InterPro | IPR008985; ConA_like_lec_gl.<br>Graphical view of domain structure. |
| ProDom | [Domain structure / List of seq. sharing at least 1 domain] |
| HOBACGEN | [Family / Alignment / Tree] |
| BLOCKS | P11439. |
| ProtoNet | P11439. |
| ProtoMap | P11439. |
| PRESAGE | P11439. |
| DIP | P11439. |
| ModBase | P11439. |
| SMR | P11439; 7B9AAD56A27C700A. |
| SWISS-2DPAGE | Get region on 2D PAGE. |
| UniRef | View cluster of proteins with at least 50% / 90% identity. |

## Keywords

**3D-structure**; **Complete proteome**; **Direct protein sequencing**; **Glycosyltransferase**; **NAD**; **Signal**; **Toxin**; **Transferase**.

## Features

 Feature table viewer

 Feature aligner

| Key | From | To | Length | Description |
|---|---|---|---|---|
| SIGNAL | 1 | 25 | 25 | |
| CHAIN | 26 | 638 | 613 | Exotoxin A. |
| DOMAIN | 26 | 277 | 252 | IA (required for target cell recognition). |
| DOMAIN | 278 | 389 | 112 | II (required for translocation in target cell cytoplasm). |
| DOMAIN | 390 | 429 | 40 | IB. |
| DOMAIN | 430 | 638 | 209 | III (required for ADP-ribosyl activity). |
| NP_BIND | 465 | 481 | 17 | NAD. |
| ACT_SITE | 578 | 578 | | |

| | | | | |
|---|---|---|---|---|
| DISULFID | 290 | 312 | | |
| CONFLICT | 4 | 4 | | T -> I (in Ref. 1). |
| CONFLICT | 22 | 22 | | F -> S (in Ref. 1). |
| CONFLICT | 204 | 204 | | A -> T (in Ref. 1). |
| CONFLICT | 389 | 389 | | S -> N (in Ref. 1). |
| CONFLICT | 432 | 432 | | I -> V (in Ref. 1). |
| CONFLICT | 540 | 540 | | G -> S (in Ref. 1). |
| STRAND | 29 | 29 | 1 | |
| HELIX | 32 | 35 | 4 | |
| STRAND | 39 | 43 | 5 | |
| TURN | 45 | 46 | 2 | |
| STRAND | 49 | 54 | 6 | |
| HELIX | 57 | 60 | 4 | |
| TURN | 61 | 61 | 1 | |
| STRAND | 65 | 74 | 10 | |
| TURN | 76 | 79 | 4 | |
| STRAND | 81 | 85 | 5 | |
| TURN | 86 | 88 | 3 | |
| STRAND | 89 | 93 | 5 | |
| STRAND | 97 | 102 | 6 | |
| STRAND | 110 | 115 | 6 | |
| STRAND | 122 | 131 | 10 | |
| TURN | 132 | 133 | 2 | |
| STRAND | 137 | 145 | 9 | |
| TURN | 147 | 148 | 2 | |
| STRAND | 151 | 154 | 4 | |
| STRAND | 157 | 161 | 5 | |
| HELIX | 164 | 170 | 7 | |
| TURN | 171 | 172 | 2 | |
| STRAND | 173 | 180 | 8 | |
| STRAND | 189 | 201 | 13 | |
| HELIX | 213 | 216 | 4 | |
| HELIX | 218 | 223 | 6 | |
| HELIX | 225 | 227 | 3 | |
| TURN | 228 | 229 | 2 | |
| HELIX | 230 | 235 | 6 | |
| HELIX | 243 | 246 | 4 | |
| TURN | 247 | 247 | 1 | |
| STRAND | 249 | 255 | 7 | |
| STRAND | 262 | 262 | 1 | |
| STRAND | 270 | 273 | 4 | |
| TURN | 276 | 277 | 2 | |
| HELIX | 280 | 290 | 11 | |
| TURN | 291 | 291 | 1 | |
| HELIX | 294 | 298 | 5 | |
| HELIX | 307 | 311 | 5 | |
| TURN | 312 | 312 | 1 | |
| HELIX | 313 | 325 | 13 | |

| | | | |
|---|---|---|---|
| TURN | 326 | 327 | 2 |
| HELIX | 330 | 332 | 3 |
| HELIX | 333 | 342 | 10 |
| TURN | 344 | 347 | 4 |
| HELIX | 348 | 356 | 9 |
| HELIX | 358 | 376 | 19 |
| TURN | 377 | 378 | 2 |
| TURN | 380 | 381 | 2 |
| HELIX | 384 | 387 | 4 |
| TURN | 388 | 389 | 2 |
| STRAND | 392 | 396 | 5 |
| HELIX | 408 | 410 | 3 |
| TURN | 411 | 412 | 2 |
| STRAND | 414 | 418 | 5 |
| HELIX | 422 | 424 | 3 |
| TURN | 436 | 437 | 2 |
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| HELIX | 444 | 456 | 13 |
| TURN | 457 | 458 | 2 |
| STRAND | 459 | 467 | 9 |
| HELIX | 469 | 477 | 9 |
| TURN | 478 | 478 | 1 |
| HELIX | 489 | 491 | 3 |
| STRAND | 494 | 497 | 4 |
| HELIX | 500 | 504 | 5 |
| TURN | 505 | 506 | 2 |
| STRAND | 508 | 508 | 1 |
| TURN | 514 | 515 | 2 |
| STRAND | 520 | 520 | 1 |
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| HELIX | 530 | 535 | 6 |
| STRAND | 536 | 538 | 3 |
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| HELIX | 548 | 556 | 9 |
| TURN | 557 | 557 | 1 |
| STRAND | 566 | 570 | 5 |
| TURN | 573 | 574 | 2 |
| STRAND | 577 | 581 | 5 |
| HELIX | 583 | 587 | 5 |
| TURN | 588 | 588 | 1 |
| STRAND | 590 | 593 | 4 |
| TURN | 600 | 601 | 2 |
| TURN | 603 | 604 | 2 |
| HELIX | 609 | 611 | 3 |
| HELIX | 614 | 617 | 4 |
| TURN | 618 | 619 | 2 |
| STRAND | 626 | 626 | 1 |

**Sequence information**

Length: **638 AA** [This is the length of the unprocessed precursor] Molecular weight: **69284 Da** [This is the MW of the unprocessed precursor] CRC64: **7B9AAD56A27C700A** [This is a checksum on the sequence]

```
        10         20         30         40         50         60
         |          |          |          |          |          |
MHLTPHWIPL VASLGLLAGG SFASAAEEAF DLWNECAKAC VLDLKDGVRS SRMSVDPAIA

        70         80         90        100        110        120
         |          |          |          |          |          |
DTNGQGVLHY SMVLEGGNDA LKLAIDNALS ITSDGLTIRL EGGVEPNKPV RYSYTRQARG

       130        140        150        160        170        180
         |          |          |          |          |          |
SWSLNWLVPI GHEKPSNIKV FIHELNAGNQ LSHMSPIYTI EMGDELLAKL ARDATFFVRA

       190        200        210        220        230        240
         |          |          |          |          |          |
HESNEMQPTL AISHAGVSVV MAQAQPRREK RWSEWASGKV LCLLDPLDGV YNYLAQQRCN

       250        260        270        280        290        300
         |          |          |          |          |          |
LDDTWEGKIY RVLAGNPAKH DLDIKPTVIS HRLHFPEGGS LAALTAHQAC HLPLETFTRH

       310        320        330        340        350        360
         |          |          |          |          |          |
RQPRGWEQLE QCGYPVQRLV ALYLAARLSW NQVDQVIRNA LASPGSGGDL GEAIREQPEQ

       370        380        390        400        410        420
         |          |          |          |          |          |
ARLALTLAAA ESERFVRQGT GNDEAGAASA DVVSLTCPVA AGECAGPADS GDALLERNYP

       430        440        450        460        470        480
         |          |          |          |          |          |
TGAEFLGDGG DISFSTRGTQ NWTVERLLQA HRQLEERGYV FVGYHGTFLE AAQSIVFGGV

       490        500        510        520        530        540
         |          |          |          |          |          |
RARSQDLDAI WRGFYIAGDP ALAYGYAQDQ EPDARGRIRN GALLRVYVPR SSLPGFYRTG

       550        560        570        580        590        600
         |          |          |          |          |          |
LTLAAPEAAG EVERLIGHPL PLRLDAITGP EEEGGRLETI LGWPLAERTV VIPSAIPTDP

       610        620        630
         |          |          |
RNVGGDLDPS SIPDKEQAIS ALPDYASQPG KPPREDLK
```

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L5: Entry 191 of 196 File: USPT Apr 15, 1997

DOCUMENT-IDENTIFIER: US 5621083 A
TITLE: Immunotoxins comprising ribosome-inactivating proteins

Brief Summary Text (8):
A variety of such gene fusions are discussed in Pastan et al., Science, 254:1173-1177 (1991). However, these fusion proteins have been constructed with sequences from diphtheria toxin or Pseudomonas aeruginosa exotoxin A, both of which are ADP-ribosyltransferases of bacterial origin. These protein toxins are reported to intoxicate cells and inhibit protein synthesis by mechanisms which differ from those of the RIPs. Moreover, diphtheria toxin and exotoxin A are structurally different from, and show little amino acid sequence similarity with, RIPs. In general, fusion proteins made with diphtheria toxin or exotoxin A have been immunogenic and toxic in animals, and are produced intracellularly in relatively low yield. Another strategy for producing a cytotoxic agent is to express a gene encoding a RIP fused to a gene encoding a targeting moiety. The resulting protein product is a single polypeptide containing a RIP linked to, for example, at least one chain of an antibody.

Detailed Description Text (198):
In the line labelled "mod", a dot (.) represents a residue which may be mutated from "mouse" to "human" at moderate risk. There are 29 such moderate risk positions.

Detailed Description Text (199):
The mouse residue matches the human consensus residue more than 50% of the time at 131 positions (102 positions match 90%-100% and 29 positions match 50% to 90%). These positions were not changed.

10223977 PMID: 7927673

**Importance of ADP-ribosylation in the morphological changes of PC12 cells induced by cholera toxin.**

Glineur C; Locht C

Unite d'Oncologie Moleculaire, CNRS URA 1160, Institut Pasteur, Lille, France.

Infection and immunity (UNITED STATES) Oct 1994, 62 (10) p4176-85, ISSN 0019-9567 Journal Code: 0246127

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Cholera toxin (CTX) is composed of two subunits, subunit A, which possesses ADP-ribosyltransferase activity, and subunit B, which is responsible for receptor binding. It has previously been shown that agents that increase cyclic AMP (cAMP) levels in cells induce differentiation of PC12 cells into neurite-like cells. In this report, we show that as little as 100 pg of CTX per ml induces such changes. CTX was found to ADP-ribosylate at least four membrane proteins of PC12 cells in vitro and in vivo and to increase intracellular cAMP levels. We have developed an inducible ctx gene expression system in Vibrio cholerae by using the tac promoter. The culture medium of the CTX-producing bacteria was able to induce the morphological changes and the ADP-ribosylation of the PC12 cell membrane proteins. We have constructed two CTX-cross-reactive mutant proteins (CTX-CRM) by site-directed mutagenesis. The choice of **glutamic** acid **29** as the target amino acid was based on sequence similarities with other bacterial toxins. CTX-CRM-E29 delta, in which the **Glu - 29** of the A subunit was **deleted** , showed strongly reduced ADP-ribosyltransferase activity and did not induce significant morphological changes of PC12 cells. In contrast, CTX-CRM-E29D, in which the **Glu - 29** was replaced by an aspartic acid, was as active as the wild-type protein. We conclude that the ADP-ribosylation activity of CTX is important for the toxin-induced differentiation of PC12 cells. Pertussis toxin, which had no visible effect on PC12 cell morphology, was also able to ADP-ribosylate a membrane-bound protein(s) in vitro and in vivo. Pertussis toxin alone did not significantly increase cAMP levels in PC12 cells, but it acted synergistically with CTX.

Tags: Support, Non-U.S. Gov't

Descriptors: Adenosine Diphosphate Ribose--metabolism--ME; * **Cholera Toxin** --toxicity--TO; Amino Acid Sequence; Animals; Base Sequence; CHO Cells; **Cholera Toxin** --biosynthesis--BI; **Cholera Toxin** --genetics--GE; Cyclic AMP--analysis--AN; Forskolin--pharmacology--PD; Genetic Vectors; Hamsters; Molecular Sequence Data; PC12 Cells--drug effects--DE; PC12 Cells--metabolism--ME; Rabbits; Rats; Recombinant Proteins--biosynthesis --BI; Recombinant Proteins--toxicity--TO

CAS Registry No.: 0 (Genetic Vectors); 0 (Recombinant Proteins); 20762-30-5 (Adenosine Diphosphate Ribose); 60-92-4 (Cyclic AMP); 66428-89-5 (Forskolin); 9012-63-9 (Cholera Toxin)

Record Date Created: 19941104

Record Date Completed: 19941104

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/06725

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/28 A61K39/00 A61K39/39 //C12N15/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 97 02348 A (BIOCINE SPA ;PIZZA MARIAGRAZIA (IT); FONTANA MARIA RITA (IT); GIAN) 23 January 1997<br>see page 4, line 10 - line 24<br>see page 5, line 31 - page 6, line 13<br>see page 8, line 35 - page 9, line 2<br>see page 15, line 33 - page 17, line 2 | 1-3,5-10 |
| Y | --- | 4 |
| Y | HÄSE C.C. ET AL.: "Construction and characterization of recombinant Vibrio cholerae strains producing inactive cholera toxin analogs"<br>INFECTION AND IMMUNITY,<br>vol. 62, no. 8, August 1994,<br>pages 3051-3057, XP002070088<br>see the whole document | 4 |
| | --- | |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 8 July 1998 | 16.07.98 |

| Name and mailing address of the ISA | Authorized officer |
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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Covone, M |

4

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 98/06725

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 5 182 109 A (TAMURA SHINICHI ET AL) 26 January 1993<br>see column 1, line 22 - line 31<br>see column 5, line 2 - line 4<br>see claims 1,3,4,6,7 | 1-3,5-10 |
| Y | HARFORD S. ET AL. : "Inactivation of the Escherichia coli heat-labile enterotoxin by in vitro mutagenesis of the A-subunit gene"<br>EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 183, no. 2, August 1989, pages 311-316, XP002070089<br>cited in the application<br>see page 315, line 43 - line 55 | 1-3,5-10 |
| P,X | WO 97 29771 A (CHIRON S P A ;FONTANA MARIA RITA (IT); PIZZA MARIAGRAZIA (IT); RAP) 21 August 1997<br>see page 4, line 6 - page 5, line 2<br>see page 45, line 16 - page 46, line 24 | 1-10 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

1: Mol Microbiol. 1996 May;20(4):823-32.



# Protein engineering studies of A-chain loop 47-56 of Escherichia coli heat-labile enterotoxin point to a prominent role of this loop for cytotoxicity.

**Feil IK, Reddy R, de Haan L, Merritt EA, van den Akker F, Storm DR, Hol WG.**

Howard Hughes Medical Institute, University of Washington, Seattle 98195-7742, USA.

Heat-labile enterotoxin (LT), produced by enterotoxigenic Escherichia coli, is a close relative of cholera toxin (CT). These two toxins share approximately 80% sequence identity, and consists of one 240-residue A chain and five 103-residue B subunits. The B pentamer is responsible for GM1 receptor recognition, whereas the A subunit carries out an ADP-ribosylation of an arginine residue in the G protein, Gs alpha, in the epithelial target cell. This paper explores the importance of specific amino acids in loop 47-56 of the A subunit. This loop was observed to be highly mobile in the inactive R7K mutant of the A subunit. The position of the loop in wild-type protein is such that it might require considerable reorganization during substrate binding and is likely to have a crucial role in substrate binding. Five single-site substitutions have been made in the LT-A subunit 47-56 loop to investigate its possible role in the enzymatic activity and toxicity of LT and CT. The wild-type residues Thr-50 and Val-53 were replaced either by a glycine or by a proline. The glycine substitutions were intended to increase the mobility of this active-site loop, and the proline substitutions were intended to decrease the mobility of this same loop by restricting the accessible conformational space. Under the hypothesis that mobility of the loop is important for catalysis, the

glycine-substitution mutants T50G and V53G would be expected to exhibit activity equal to or greater than that of the wild-type A subunit, while the proline substitution mutants T50P and T53P would be less active. Cytotoxicity assays showed, however, that all four of these mutants were considerably less active than wild-type LT. These results lend support for assignment of a prominent role to loop 47-56 in catalysis by LT and CT.

PMID: 8793878 [PubMed - indexed for MEDLINE]

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Feil IK, et al.
Mol Microbiol. 1996 May;20(4):823-32.
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van den Akker F, et al.
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3: Stepwise transplantation of an active site loop between heat-labile enterotoxins LT-II and LT-I and characterization of the obtained hybrid toxins. Related Articles, Cited in PMC, Books, Linl
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4: Glutamic acid-112 of the A subunit of heat-labile enterotoxin from enterotoxigenic Escherichia coli is important for ADP-ribosyltransferase activity. Related Articles, Cited in PMC, Books, Linl
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Lobet Y, et al.
Infect Immun. 1991 Sep;59(9):2870-9.
PMID: 1908825 [PubMed - indexed for MEDLINE]

13125469 PMID: 8793878

Protein engineering **studies of A-chain loop 47-56 of Escherichia coli heat-labile enterotoxin point to a prominent role of this loop for cytotoxicity.**

**Feil I K** ; Reddy R; de Haan L; Merritt E A; van den Akker F; Storm D R; Hol W G

Howard Hughes Medical Institute, University of Washington, Seattle 98195-7742, USA.

Molecular microbiology (ENGLAND) May **1996** , 20 (4) p823-32, ISSN 0950-382X Journal Code: 8712028

Contract/Grant No.: AI34501; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Heat-labile enterotoxin (LT), produced by enterotoxigenic Escherichia coli, is a close relative of cholera toxin (CT). These two toxins share approximately 80% sequence identity, and consists of one 240-residue A chain and five 103-residue B subunits. The B pentamer is responsible for GM1 receptor recognition, whereas the A subunit carries out an ADP-ribosylation of an arginine residue in the G **protein** , Gs alpha, in the epithelial target cell. This paper explores the importance of specific amino acids in loop 47-56 of the A subunit. This loop was observed to be highly mobile in the inactive R7K mutant of the A subunit. The position of the loop in wild-type **protein** is such that it might require considerable reorganization during substrate binding and is likely to have a crucial role in substrate binding. Five single-site substitutions have been made in the LT-A subunit 47-56 loop to investigate its possible role in the enzymatic activity and toxicity of LT and CT. The wild-type residues Thr-50 and Val-53 were replaced either by a glycine or by a proline. The glycine substitutions were intended to increase the mobility of this active-site loop, and the proline substitutions were intended to decrease the mobility of this same loop by restricting the accessible conformational space. Under the hypothesis that mobility of the loop is important for catalysis, the glycine-substitution mutants T50G and V53G would be expected to exhibit activity equal to or greater than that of the wild-type A subunit, while the proline substitution mutants T50P and T53P would be less active. Cytotoxicity assays showed, however, that all four of these mutants were considerably less active than wild-type LT. These results lend support for assignment of a prominent role to loop 47-56 in catalysis by LT and CT.

Tags: Female; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Bacterial Toxins--chemistry--CH; *Enterotoxins--chemistry --CH; Animals; Cricetulus; Cyclic AMP--metabolism--ME; Enzyme-Linked Immunosorbent Assay; Escherichia coli; Hamsters; Mutagenesis, Site-Directed ; Ovary--drug effects--DE; Ovary--metabolism--ME; **Protein** Conformation; Structure-Activity Relationship

CAS Registry No.: 0 (Bacterial Toxins); 0 (Enterotoxins); 0 (enterotoxin LT); 60-92-4 (Cyclic AMP)

Record Date Created: 19970220

Record Date Completed: 19970220